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Carbonic anhydrase inhibition and antioxidant activity of the axially naphthoxazin group substituted silicon phthalocyanines

Eksenel olarak naftoksazin grubu sübstitüe edilmiş silisyum ftalosiyaninlerin karbonik anhidraz inhibisyonu ve antioksidan aktivitesi

Ayça AKTAŞ KARAÇELİK*[1,a], Volkan ÇAKIR[2,b], Hüseyin BAŞ[3,c], Zekeriya BIYIKLIOĞLU[3,d]

[1] Giresun University, Espiye Vocational School, Department of Food Processing, 28600, Giresun
[2]Giresun University, Espiye Vocational School, Department of Therapy and Rehabilitation, 28600, Giresun
[3]Karadeniz Technical University, Faculty of Sciences, Department of Chemistry, 61000, Trabzon

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Abstract
Silicon phthalocyanines are an interesting subclass of phthalocyanines. They are abundant and have extremely low toxicity levels. The low solubility of silicon phthalocyanine is the major obstacle to its use in many different applications. Therefore, in a previous study, two axially substituted silicon phthalocyanines were synthesized to increase their solubility. In this study, these axially substituted silicon phthalocyanines were evaluated for carbonic anhydrase inhibition and antioxidant activities. The carbonic anhydrase (CA) inhibition potential of silicon phthalocyanines was evaluated by esterase activity. The antioxidant activity was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric ion (III) reducing/antioxidant power (FRAP) assays. The silicon phthalocyanines had significant CA inhibitory activity [50% inhibitory values (IC_{50}): 495 ± 12.74 nM and 857 ± 13.03 nM for H4-Si and H3-Si, respectively]. According to the antioxidant studies, 50% scavenging concentration (SC_{50}) values of DPPH• assay were 2.29 ± 0.06 µg/mL and 1.39 ± 0.43 µg/mL for H3-Si and H4-Si and Trolox Equivalent Antioxidant Capacity (TEAC) values of FRAP test were 259.33 ± 48.27 µM and 342.00 ± 44.40 for H3-Si µM and H4-Si, respectively. Consequently, silicon phthalocyanine compounds are considered to have great potential for their use in various fields such as food and medicine.

Keywords: Antioxidant, Carbonic anhydrase, Esterase activity, Inhibitor, Silicon phthalocyanines

Öz
Silisyum ftalosiyaninler, ftalosiyaninlerin ilgi duyulan bir alt sınıfıdır. Bol miktarda bulunurlar ve son derece düşük toksisite seviyelerine sahiptirler. Silisyum ftalosiyanininin düşük çözünürlüğü, birçok farklı uygulamada kullanımının önündeki en büyük engeldir. Bu yüzden, çözünürlüklerini artırmak için eksenel olarak sübstitüe edilmiş iki silisyum ftalosiyanin daha önce yapılan bir çalışmada sentezlendi. Bu çalışmada, eksenel olarak sübstitüe edilmiş bu silisyum ftalosiyaninler, karbonik anhidraz inhibisyonu ve antioksidan aktiviteleri açısından değerlendirildi. Silisyum ftalosiyaninlerin karbonik anhidraz (CA) inhibisyon potansiyeli, esteraz aktivitesi ile değerlendirildi. Antioksidan aktivite, 2,2-difenil-1-pikrilhidrazil (DPPH•) radikal temizleme ve demir iyon (III) indirgeme / antioksidan güç (FRAP) metotları ile test edildi. Silisyum ftalosiyaninler, önemli CA inhibitör aktivitesine [%50 inhibitör değerleri (IC_{50}): H4-Si ve H3-Si için sırasıyla 495 ± 12.74 nM ve 857 ± 13.03 nM] sahipti. Antioksidan çalışmalara göre, DPPH• testinin %50 temizleme konsantrasyon (SC_{50}) değerleri sırasıyla H3-Si için 2.29 ± 0.06 µg/mL, H4-Si için 1.39 ± 0.43 µg/mL ve FRAP testinin Trolox Eşdeğer Antioksidan Kapasitesi (TEAC) değerleri H3-Si için 259.33±48.27 µM, H4-Si için 342.00 ± 44.40 µM olarak bulundu. Sonuç olarak, silisyum ftalosiyanin bileşiklerinin, gıda ve tıp gibi çeşitli alanlarda kullanımları için büyük potansiyele sahip olduğu düşünülmektedir.

***Anahtar kelimeler**: Antioksidan, Karbonik anhidraz, Esteraz aktivitesi, İnhibitör, Silisyum ftalosiyaninler*

[*a] Ayça AKTAŞ KARAÇELİK; ayca.aktas@giresun.edu.tr, Tel: 0 (454) 310 14 30, orcid.org/0000-0001-5381-2924
[b] orcid.org/0000-0002-5817-0817 [c] orcid.org/0000-0002-8722-3359 [d] orcid.org/0000-0001-5138-214X

1. Introduction
1. Giriş

Phthalocyanines (Pc) are functional macro/heterocyclic dyes with planar structure with thermal and optic stability due to a strong 18-π electron conjugation. Phthalocyanines derivatives have become increasingly important in many application fields such as dyes and pigments (Leznoff & Lever, 1996), medical applications, and photodynamic therapy (Rodriguez et al., 2009; Master et al., 2010). In addition to their unique electronic, optical, and structural behaviors other properties have been explored such as antifungal, antibacterial, and antitumor activities (Zhao et al., 2013). However, unsubstituted Pcs have obstacles to their application because of their insolubility. It was discovered that there are several approaches to introduce a suitable substituent on the ring system of Pcs which increase the steric interactions. Suitable substituent includes electron-withdrawing (–F, –Cl, –Br, $–NO_2$, etc.), electron-donating ($–NH_2$, Ar–S–, RO–, etc.), and bulky or long-chain groups which help solve the low solubility issue (Çakir et al., 2015). Phthalocyanines aggregation in solution is another limitation. Fortunately, recent researches demonstrated that axially disubstituted silicon phthalocyanines (SiPcs) displayed non-aggregation due to the non-planar substituents issuing from the center metal atom (Demirkapi et al., 2014; Güzel et al., 2019). To overcome this problem, it is preferable to add substituents to the axial positions of Pcs, as axial positions can strongly affect some properties of Pcs such as solubility and aggregation behavior. Non-aggregating axially disubstituted SiPcs can be used for biological activity determination applications. Previous researches have also shown that the toxicity of SiPc against cancer cells is very low (Chan et al., 2010; Li et al., 2017; Bispo et al., 2018). Therefore, the synthesis of an axially substituted SiPc would be a good candidate for further study.

Carbonic anhydrase (EC 4.2.1.1. CA) have vital metabolic and cellular functions such as carbon dioxide and ion transport, acid-base balance, respiration, homeostasis, bone resorption, gluconeogenesis, ureagenesis, lipogenesis, tumorigenesis, and electrolyte secretion (Supuran & Scozzafava, 2002; Supuran et al., 2003). Although CA has important metabolic and cellular functions, it is common in the medical field to inhibit CAs in order to treat several disorders such as glaucoma, edema, epilepsy, hypoxic tumors, and obesity (Supuran, 2011). Therefore, finding novel CA inhibitors is of great interest as is finding novel antioxidants compounds.

Reactive oxygen species (ROS) are implicated in causing aging, cancer, and other disorders (hepatic, neurodegenerative, cardiovascular, and renal) in humans. Oxidative stress is the result of an imbalance between ROS and antioxidant defenses. It can cause various disorders (cancer, cardiovascular diseases, neurodegenerative disorders, diabetes, etc.) and degeneration on cell functions resulting in cell death. Antioxidants are one of the most important compounds used to protect living organisms because of their ability to inhibit ROS (Karaçelik et al., 2015; Alkan Türkuçar et al., 2021). Synthesis and characterization of new phthalocyanine compounds with different functional groups with bioactive properties have been investigated (Yıldırım et al., 2017; Karaçelik et al., 2021a). In addition, in recent years, there has been an increasing interest in these compounds, as phthalocyanine molecules exhibit antioxidant and enzyme inhibition properties according to the metal ion and substituted groups in their center (Demirkapi et al., 2014; Agirtaş et al., 2018; Güzel et al., 2018; Günsel et al., 2019; Demirbaş et al., 2019; Özil et al., 2019; Yakan et al., 2020; Karaçelik et al., 2021; Aktaş Karaçelik et al., 2021).

In the light of this information, it was aimed to evaluate the CA inhibition activity and antioxidant activity of two axially substituted silicon phthalocyanines (H3-Si and H4-Si) compounds.

2. Materials and methods
2. Materyal ve metot

2.1. Chemical synthesis
2.1. Kimyasal sentez

2-[2-(1H-naphtho[1,2-e][1,3]oxazin-2(3H)-yl)ethoxy]ethanol and 6-(1H-naphtho[1,2-e][1,3]oxazin-2(3H)-yl)hexan-1-ol axially naphthoxazin substituted two silicon(IV) phthalocyanines were prepared according to Baş and Biyiklioglu (2015). The IR spectra were determined using a Perkin Elmer 1600 Fourier Transform-Infrared (FT-IR-ATR) spectrophotometer. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer in DMSO-d6, and chemical shifts (δ) are reported in ppm and coupling constants (J) are given in hertz (Hz). Mass spectra were measured on a Bruker Microflex LT MALDI-TOF MS spectrometer. Melting points

were measured on a Barnstead electrothermal 9200 series digital apparatus. Electronic spectra in the UV–Vis region were recorded on a Perkin Elmer-Lambda 25 spectrophotometer, using 1 cm pathlength cuvettes at room temperature. Silicon (IV) phthalocyanine dichloride was purchased from Sigma Aldrich. All reagents were of reagent grade quality and obtained from commercial suppliers. All solvents were dried and purified as described by Perrin and Armarego (1985).

2.2. Determination of CA inhibition activity
2.2. CA inhibisyon aktivitesinin belirlenmesi

Esterase activity was used to evaluate CA inhibition of SiPcs (H3-Si and H4-Si) (Verpoorte et al., 1967). Esterase activity reactions were performed according to the method previously described by Efe (2020). bCA enzyme was commercially obtained from Sigma Aldrich. Enzyme activity was spectrophotometrically determined by measuring the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) to *p*-nitrophenol or *p*-nitrophenolate at 348 nm wavelength. The inhibitory effects of H3-Si and H4-Si were determined at the following different concentrations: 0.02-0.004 mg/mL for H3-Si and 0.025-0.008 mg/mL for H4-Si. The negative control was prepared with the same reaction mixture without an enzyme. The positive control was prepared with the reaction mixture with the enzyme in the absence of the inhibitor. The results were given as inhibitor concentrations (IC_{50}) resulting in 50% inhibition. All experiments were performed in triplicate.

2.3. Determination of ferric reducing / antioxidant power (FRAP)
2.3. Demir indirgeme / antioksidan gücün (FRAP) belirlenmesi

The method of FRAP was based on the spectrophotometric measurement of the revealed TPTZ-Fe (II) complex as a reaction product (Benzie & Strain, 1996). Trolox Equivalent Antioxidant Capacity (TEAC) was determined for H3-Si and H4-Si using the calibration graph of Trolox (31.25-1000 µM). Briefly, a 50 µL sample (at 0.04 mg/mL, corresponding to 36.86 and 36.03 µM for H3-Si and H4-Si) was mixed with 1.5 mL of FRAP reagent and spectrophotometrically measured at 595 nm wavelength (Karaçelik et al., 2015). All experiments were performed in triplicate.

2.4. Determination of DPPH• radical scavenging activity
2.4. 2.4. DPPH• radikal temizleme aktivitesinin belirlenmesi

2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to determine radical scavenging activity (Brand-Williams et al., 1995). SiPcs (H3-Si and H4-Si) were evaluated for DPPH• radical scavenging activity according to the method described by Karaçelik (2021). Serial dilutions of H3-Si and H4-Si were mixed with 100 µM methanolic DPPH• solution in an equal volume (750 µL) and spectrophotometrically measured at 517 nm wavelength after 50 min incubation. The results were given as the SC_{50} (the compound concentration showing 50% scavenging of DPPH• initially available, µg/mL) value. All experiments were performed in triplicate. Also, %DPPH• scavenging activity at 0.0025 mg/mL for each sample and standard was calculated.

3. Results and discussion
3. Bulgular ve tartışma

3.1. Chemical synthesis
3.1. Kimyasal sentez

2- [2- (1H- naphtho[1,2-e][1,3] oxazin-2(3H)-yl) ethoxy]ethanol and 6-(1H-naphtho[1,2 e][1,3]oxazin-2(3H)-yl)hexan-1-ol axially naphthoxazin substituted two silicon(IV) phthalocyanines (SiPcs) were previously synthesized (Baş & Biyiklioglu, 2015). Axially naphthoxazin group substituted SiPcs are shown in Figure 1.

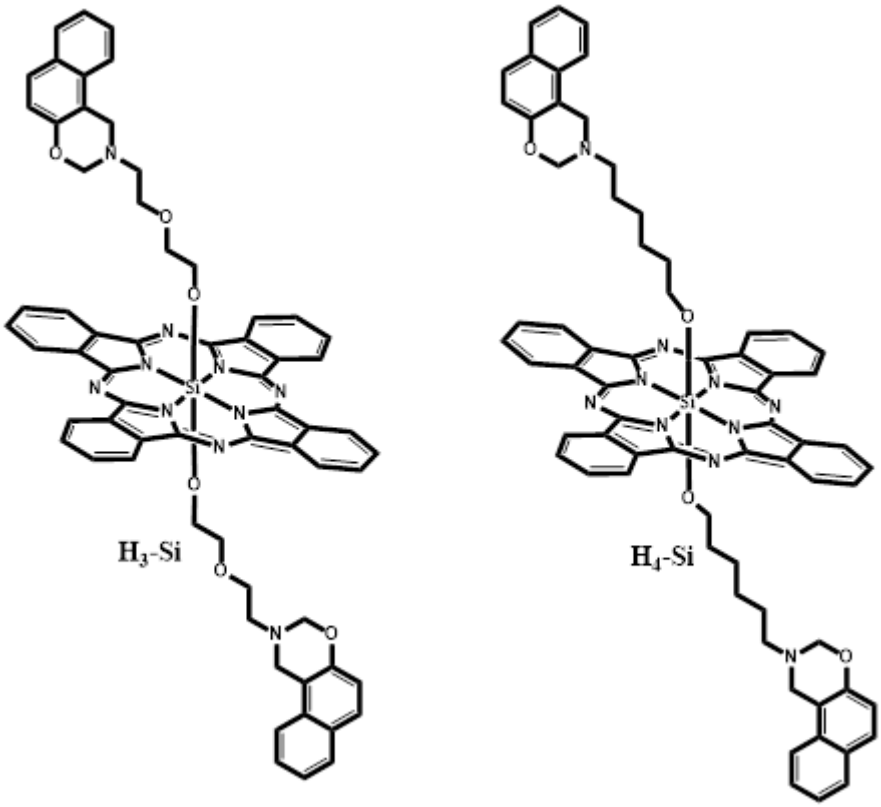

Figure 1. Axially naphthoxazin group substituted silicon phthalocyanines (Baş & Biyiklioglu, 2015)
Şekil 1. *Eksenel naftoksazin grubu sübstitüe silisyum ftalosiyaninler (Baş ve Biyiklioğlu, 2015)*

3.2. Carbonic anhydrase inhibition and antioxidant activity
3.2. Karbonik anhidraz inhibisyonu ve antioksidan aktivite

Carbonic anhydrase inhibition activity of SiPcs was tested on bovine carbonic anhydrase (bCA) by esterase activity *in vitro*. The determined CA inhibition potentials were given as IC_{50} values in Figure 2 and Table 1. According to the bCA inhibition activity assay, IC_{50} was 495±12.74 nM for H4-Si and 857±13.03 nM for H3-Si. The tested SiPcs exhibited much better inhibition activity than sulfanilamide, as a low IC_{50} value indicated a high CA inhibition activity. In our previous study, ([3-(diethylamino)phenoxy]propanoxy) substituted silicon phthalocyanine showed CA inhibitory activity with an IC_{50} value of 2333 nM. However, the compounds (H3-Si and H4-Si) in this study showed approximately 2.7 and 4.7 times higher CA inhibitory activity, respectively (Aktaş Karaçelik et al., 2021). Consequently, these compounds had great potential as CA inhibitors and could have significant importance in the find new classes of potential CA inhibitors. In our previous study, it was found that metallophthalocyanines have a strong inhibitory effect on the carbonic anhydrase enzyme (Karaçelik et al., 2021). The results revealed that silicon phthalocyanines with different functional groups considerably affected biological activity. Although there has been ample research related to the inhibition activity of PCs on different enzymes, there has been only scant research about the CA inhibition activity of SiPcs (Kantar et al., 2016; Barut et al., 2017a; Arslan et al., 2019; Demirbaş et al., 2019). Arslan et al. (2018) reported that SiPcs had the potential to be carbonic anhydrase inhibitors for the first time. In another research, Keleş et al. (2019) showed that the silicon (IV) phthalocyanine and napthalocyanine containing pyridine groups had inhibitory effects on topoisomerases. In addition, water-soluble and morpholine disubstituted SiPcs were revealed to be a promising photo-synthesizing agent for photodynamic therapy due to their DNA photocleavage properties (Barut et al., 2017b; Keleş et al., 2019).

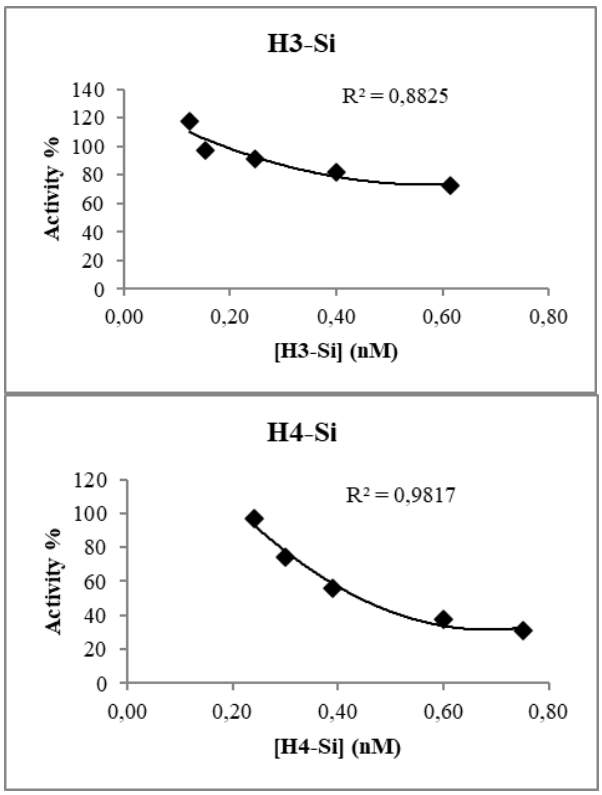

Figure 2. IC_{50} graphs of H3-Si and H4-Si phthalocyanines against bCA enzyme
Şekil 2. *H3-Si ve H4-Si ftalosiyaninlerin bCA enzimine karşı IC_{50} grafikleri*

Antioxidants decreasing the toxic influences of free radicals can be synthetic or natural compounds. Recently, investigations focused on discovering promising PCs with antioxidant activity. In this study, the antioxidant activity of H3-Si and H4-Si compounds were determined using DPPH• and FRAP assays (Table 1). According to results of the DPPH• radical scavenging activity, SC_{50} was 2.29 ± 0.06 µg/mL for H3-Si and 1.39 ± 0.43 µg/mL for H4-Si. It can be concluded that H4-Si exhibited approximately 1.7 times higher activity than H3-Si since a low SC_{50} value indicates higher antioxidant activity. In addition, both SiPcs had higher antioxidant activity than the standards (BHT, Trolox, and Vitamin C). The results of FRAP were in accordance with the DPPH• assay results. The FRAP value of H4-Si (342.00 ± 44.40 µM TEAC) was higher than the FRAP value of H3-Si (259.33 ± 48.27 µM TEAC). Both SiPcs at the tested concentrations exhibited 7.02-9.49 times higher activity than Trolox (Table 1). Their %DPPH• radical scavenging activity was calculated to be 2.5 µg/mL. The results showed that the antioxidant activities for both SiPcs (53.71% for H3-Si and 94.43% for H4-Si), were more effective than the standards (33.80% Trolox, 14.87% BHT, and 4.24% Vitamin C). These results of the study were similar to our previous study in the literature (Aktaş Karaçelik et al., 2021). Importantly, although there has been ample research related to the antioxidant activity of PCs, there is only our study on the antioxidant activity of SiPcs (Aktaş Karaçelik et al., 2021). The compounds in this study showed higher antioxidant activity compared to the antioxidant activity results of the previously studied axially substituted silicon phthalocyanines (DPPH• SC_{50} values: DM-C3-Si: 19.9 µg/mL and DE-C3-Si: 23.2 µg/mL, FRAP TEAC values: DM-C3-Si: 232 µM and DE-C3-Si: 73 µM) (Aktaş Karaçelik et al., 2021). When antioxidant activity results of this study were compared with the previously tested PCs within the literature, the SiPcs were in agreement with the literature and even had better antioxidant activity than some PCs (Agirtaş et al., 2019; Günsel et al., 2019; Unluer et al., 2019; Günsel et al., 2020).

Table 1. CA enzyme inhibition and antioxidant activity values of silicon phthalocyanines and standards
Tablo 1. *Silikon ftalosiyaninler ve standartların CA enzim inhibisyonu ve antioksidan aktivite değerleri*

Samples and standards	Carbonic anhydrase inhibition	Antioxidant activity		
	IC_{50} (nM)	DPPH• scavenging (SC_{50}, µg/mL)	%DPPH• scavenging (at 0.0025 mg/mL)	FRAP* (TEAC, µM)
H3-Si	857±13.03	2.29±0.06	53.71	259.33±48.27
H4-Si	495±12.74	1.39±0.43	94.43	342.00±44.40
Trolox	NT	3.91±0.01	33.80	**
BHT	NT	8.11±0.16	14.87	NT
Vitamin C	NT	28.77±1.01	4.24	NT
Sulfanilamide	5030.56±59.88	NT	NT	NT
Acetazolamide	80.01±0.33	NT	NT	NT

* The compounds were tested at 0.04 mg/mL, corresponding to 36.86 and 36.03 µM for H3-Si and H4-Si, respectively. NT: Not tested. **Trolox was used to constructing a calibration curve used for the calculation of TEAC values.

4. Conclusion
4. Sonuç

In this study, the previously synthesized SiPcs were tested for carbonic anhydrase inhibition and antioxidant activity. According to our enzyme inhibition and antioxidant activity results, these compounds have great potential to be used as active pharmaceutical and antioxidant agents. The SiPcs studied had much better %DPPH• scavenging activity than Vitamin C. Especially, it was observed that H4-SiPc would be a good candidate to be used in the treatment of various diseases in the future because of its significant enzyme inhibition and antioxidant activity. However, it is certain that further studies (*in vivo*) are needed to produce new carbonic anhydrase inhibitors with drug potential.

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Author contribution
Yazar katkısı

Ayça AKTAŞ KARAÇELİK: Analysis and evaluation of CA inhibition and antioxidant activity.
Volkan ÇAKIR, Hüseyin BAŞ, Zekeriya BIYIKLIOĞLU: Synthesis of chemicals and characterized.
Authors contributed equally to this work.

Declaration of ethical code
Etik beyanı

The authors of this article declare that the materials and methods used in this study do not require ethical committee approval and/or legal-specific permission.

Conflicts of interest
Çıkar çatışması

The authors declare that there is no conflict of interest.

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